


# An autoparasitoid wasp, inferior at resource exploitation, outcompetes primary parasitoids by using competitor females to produce males

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**Abstract.** 1. Autoparasitoids are intraguild consumers that attack and kill heterospecific and conspecific parasitoids as well as immature stages of hemipteran hosts, such as aphids, whiteflies and soft scales. Field experiments assessing the importance of interspecific competition between autoparasitoids and primary parasitoids, as well as its impact on herbivore suppression, are scarcely found in the ecological literature.

2. Using field data from 40 olive orchards, this study examined the mechanisms that regulate: (i) the interspecific competition between primary parasitoids of the genus *Metaphycus* and the autoparasitoid *Coccophagus lycimnia*; and (ii) the density of their shared herbivore host, the soft scale *Saissetia oleae*.

3. *Metaphycus* parasitoids used smaller hosts than *C. lycimnia*, yet did not outcompete *C. lycimnia*. On the other hand, *C. lycimnia* preferred to use *Metaphycus* females as secondary hosts for producing males rather than their own females. This preference might explain why the autoparasitoid negatively affected the density of the primary parasitoids.

4. Parasitism by the autoparasitoid *C. lycimnia* at the beginning of the season was the sole variable positively related to host mortality throughout the season, showing its greater effect on herbivore suppression.

5. In this study, an autoparasitoid, inferior at resource exploitation, was shown to outcompete a primary parasitoid without disrupting herbivore suppression.

**Key words.** *Coccophagus*, exploitative competition, interspecific competition, intraguild predation, *Metaphycus*, soft scales.

## Introduction

Historically, attempts at biological control have often failed because they ignored the fact that natural enemies are part of a complex food web, where the top-down regulation of herbivore populations is the outcome of multiple species interacting at different trophic levels (Murdoch *et al.*, 1985; Rosenheim *et al.*, 1995; Rosenheim, 1998; Borer *et al.*, 2003, 2004; Janssen *et al.*, 2006; Boivin & Brodeur, 2006; Tougeron & Tena, 2019). A common interaction in these multitrophic food webs is intraguild predation, wherein one natural enemy consumes

another. Numerous studies have suggested that intraguild predation weakens the top-down regulation of herbivores by natural enemies (May & Hassell, 1981; Polis *et al.*, 1989; Rosenheim *et al.*, 1995; Rosenheim, 1998; Borer *et al.*, 2003; Boivin & Brodeur, 2006; Janssen *et al.*, 2006). Hymenopteran parasitoids are often among the most important natural enemies of herbivores, but they are commonly victims rather than perpetrators of intraguild predation because their larvae are susceptible to consumption by competing predators that feed on the same herbivorous hosts (Rosenheim *et al.*, 1995; Rosenheim, 1998; Snyder & Ives, 2008; Frago, 2016). However, some species of parasitoids can also act as intraguild attackers. In some species, males develop as obligate hyperparasitoids of females of their own and other parasitoid species, whereas females develop as primary parasitoids of herbivores, i.e. autoparasitoid *sensu*

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(Hunter & Woolley, 2001). Therefore, like intraguild predators, autoparasitoids can kill and consume both their competitors and their common hosts (Rosenheim *et al.*, 1995).

The impacts of autoparasitoids in mediating the strength of top-down regulation of herbivores by their primary parasitoids sparked a lively debate in the literature of the 1990s and 2000s. A key question was whether any suppression of herbivore regulation caused by parasitism of primary parasitoids could be compensated by the autoparasitoids' direct parasitism on herbivorous hosts. From a theoretical perspective it is difficult to anticipate whether an autoparasitoid will cause an increase or decrease in long-term herbivore population densities when the main entomophagous arthropod is a primary parasitoid (Snyder & Ives, 2008). This is because, based on theory, the disruption of host suppression by the autoparasitoid requires moderately strong but not too strong attack rates on hosts parasitised by the primary parasitoid (Snyder & Ives, 2008). Therefore, according to theoretical studies, the outcome of competition between primary parasitoids and autoparasitoids for host suppression variably falls between disruption and synergism.

Studies examining the effect of autoparasitoids on host suppression have reached divergent conclusions (Bográn *et al.*, 2002; Hunter *et al.*, 2002). These studies mostly comprised facultative autoparasitoids of the genus *Encarsia* (Hymenoptera: Aphelinidae) parasitising whiteflies (Hemiptera: Aleyrodidae) in field cages. Hunter *et al.* (2002) examined the interactions among the autoparasitoid *Encarsia sophia* [= *E. transvena* (Timberlake)], the primary parasitoid *Eretmocerus eremicus* Rose and Zolnerowich (Hymenoptera: Aphelinidae) and their shared host, the sweet potato whitefly *Bemisia tabaci* (Genadius) (Hemiptera: Aleyrodidae). The interference by the autoparasitoid reduced primary parasitoid density, but produced no concomitant disruption of host suppression. The results support theoretical predictions that no disruption should occur when both parasitoids are equally efficient and suggest that an autoparasitoid may be as efficient as a primary parasitoid in suppressing herbivore populations (Snyder & Ives, 2008). Bográn *et al.* (2002) examined interactions among three parasitoids of the silverleaf whitefly *Bemisia argentifolii* Bellows and Perring (Hemiptera: Aleyrodidae): the autoparasitoid *Encarsia pergandiella* Howard and the primary parasitoids *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae) and *Encarsia formosa* Gahan. Contrary to the previous example, the autoparasitoid *E. pergandiella* disrupted whitefly suppression. In addition, the study demonstrated that primary parasitoids could also reduce the population density of autoparasitoids through asymmetric exploitative competition, because the primary parasitoid *E. mundus* utilises younger developmental stages of the host than the autoparasitoid *E. pergandiella*. Mathematical simulations also predicted that parasitoid species attacking early host stages will always outcompete a parasitoid attacking later stages of the same host, unless the later attacking species is able to use previously parasitised hosts (Briggs, 1993). Briggs' (1993) theoretical predictions were partially supported by experimental work by Bográn *et al.* (2002) as the earlier host-stage-attacking *E. mundus* affected the population growth rates of both *Encarsia* species. While the previous field studies have contributed greatly to our understanding

of the competition between primary parasitoids and autoparasitoids, their contribution to understanding the population dynamics of the insects involved lacked validation in natural scenarios because parasitoids were artificially released in cages, so their phenology and any effect of metapopulation structure, among other variables, were not considered (Janssen *et al.*, 2006).

*Coccophagus* (Hymenoptera: Aphelinidae) is a common genus of autoparasitoids. Parasitoids of this genus have been widely used in several programmes of biological control against soft scale insects (Hemiptera: Coccidae) together with primary parasitoids of the *Metaphycus* (Hymenoptera: Encyrtidae) genus (Kapranas & Tena, 2015). The latter are considered the main natural enemies of soft scales because unlike *Coccophagus* they are strictly primary parasitoid and can exploit smaller scales and hence can capitalise hosts through exploitative competition. However, it is unclear whether *Coccophagus* can negatively influence the population dynamics of *Metaphycus* and consequently the population density of soft scale insects (Kapranas & Tena, 2015). Excessive hyperparasitism by *Coccophagus lycimnia* (Walker) (Hymenoptera: Aphelinidae) has been suggested as one factor preventing *Metaphycus helvolus* (Compere) (Hymenoptera: Encyrtidae) populations from building up as host density increased, resulting in poor control of *Coccus pseudomagnoliarum* (Kuwana) (Hemiptera: Coccidae) in Californian citrus (Bernal *et al.*, 1998, 1999, 2001). However, later studies suggested that *Coccophagus* species could be efficient biological control agents of soft scale insect pests because they can reach greater population densities than *Metaphycus* (Schweizer *et al.*, 2002, 2003). Similarly, an increase in *Coccophagus* parasitism on *Coccus hesperidum* L. (Hemiptera: Coccidae) did not lead to a reduction of parasitism by *Metaphycus* species (Kapranas *et al.*, 2007). While these observations are based on field surveys, research on the role of interspecific competition between primary parasitoids and autoparasitoids on their own population dynamics and that of their shared coccid hosts is still lacking. These types of studies are needed to reconcile theory with extensive observations from agricultural systems.

Here, we report data on insects sampled at 40 olive orchards where several primary parasitoids of the *Metaphycus* genus and one autoparasitoid, *C. lycimnia*, coexist simultaneously and regulate the black scale *Saissetia oleae* (Olivier) (Hemiptera: Coccidae) (Pereira, 2004). These data were used to analyse the mechanisms that regulate the interspecific competition between primary parasitoids and an autoparasitoid, as well as whether this competition affects the density of their shared coccid host. Regarding the mechanisms, we first hypothesise that small hosts provide competition-free space for the primary parasitoids. Strong host exploitation by the facultative autoparasitoid species should, therefore, lead to a shift in the size of the hosts attacked by the primary parasitoids. More precisely, we hypothesise that *Metaphycus* should develop on smaller hosts under conditions of high host exploitation by the autoparasitoid *C. lycimnia*. Second, we (indirectly) tested whether *C. lycimnia* has a sex determination strategy that responds to the density of the primary parasitoids. In particular, we hypothesise that the adult (secondary) sex ratio of *C. lycimnia* will be increasingly male-biased as a

higher percentage of hosts are exploited by primary parasitoids. Our study is novel because prior field studies did not examine the role of interspecific competition between primary parasitoids and an autoparasitoid, their population dynamics, and the population dynamics of their shared host.

## Materials and methods

### Study system

*Saissetia oleae* is a serious pest of citrus and olive in most regions of the world (Ben-Dov & Hodgson, 1997). This pestiferous insect is oviparous and parthenogenetic. It has one annual generation, although in the studied region a second partial generation is possible in olive trees (Pereira, 2004; Tena *et al.*, 2007). Eggs are laid at the end of spring and in autumn when the second generation occurs. The generations are highly synchronised (Tena *et al.*, 2007). This species has three immature nymphal instars. The first instar, also known as the crawler, is mobile and is not attacked by parasitoids. The second and third instars are the predominant instars from September to May and they tend to settle on leaves (Pereira, 2004; Tena *et al.*, 2007). There, they are attacked by a complex of parasitoids dominated by the genus *Metaphycus* and *Coccophagus*. Adults of coccid are attacked by the parasitoid *Metaphycus lounsburyi* (Howard) (Hymenoptera: Encyrtidae). *Saissetia oleae* oviposition occurs predominantly from the end of May to July, the eggs of which are attacked by the egg predator *Scutellista caerulea* (Fonscolombe) (Hymenoptera: Pteromalidae) (Pereira, 2004; Tena *et al.*, 2008a).

Herein, we studied the interaction of the primary parasitoids of the *Metaphycus* genus and the autoparasitoid *C. lycimnia* when they parasitise the immature instars of *S. oleae*. The three species of *Metaphycus* [*M. helvolus*, *M. flavus* (Howard) and *M. lounsburyi*] were included in the research. The two former species are the main parasitoids of *S. oleae* nymphs in the Mediterranean region (Pereira, 2004; Tena *et al.*, 2008a), whereas *M. lounsburyi* is often considered specific to adult scales, even though it has also been recovered from immature instars (Pereira, 2004; Tena *et al.*, 2008a; Tena & Garcia-Marí, 2009). The three species are facultatively gregarious endoparasitoids (generally a single egg is laid inside the host but not always), arrhenotokous (unfertilised eggs produce males and fertilised eggs produce females), and synovigenic (females emerge with at most a small fraction of their lifetime complement of mature eggs). In addition, *M. helvolus* and *M. flavus* exhibit destructive host-feeding; adults can kill their hosts for nutrition, not only use them for reproduction (Flanders, 1942; De Bach, 1943; Bernal *et al.*, 1999; Kapranas & Luck, 2008; Tena *et al.*, 2008b). See Guerrieri and Noyes (2000) and Kapranas and Tena (2015) for more information on biology, taxonomy, and geographical distribution. *Coccophagus lycimnia* is an autoparasitoid whose males develop as obligate hyperparasitoids of both conspecific and heterospecific females, while females develop in immature *S. oleae* scales. This parasitoid is solitary, arrhenotokous, and can also host feed (Muegge & Lambdin, 1989; Bernal *et al.*, 2001).

### Orchards and experimental design

To determine the intensity of the competition between the primary parasitoids of the *Metaphycus* genus and the autoparasitoid *C. lycimnia* and how this competition affects natural populations of their common host, *S. oleae*, 40 olive orchards with distinct host densities at the beginning of the season (September) were selected.

The 40 orchards were located in the olive-growing region of Trás-os-Montes, northeast Portugal (Fig. 1). The characteristics of the orchards are detailed in Appendix S1. Briefly, orchards had two soil cover types: bare soil by means of herbicide applications or tilling and natural ground vegetation. The planting pattern varied between 6 × 6 m and 10 × 10 m. Olive trees were between 40 to 50 years old, were pruned every 2–3 years, and were not irrigated. During the assay the orchards were under full commercial production without insecticide use.

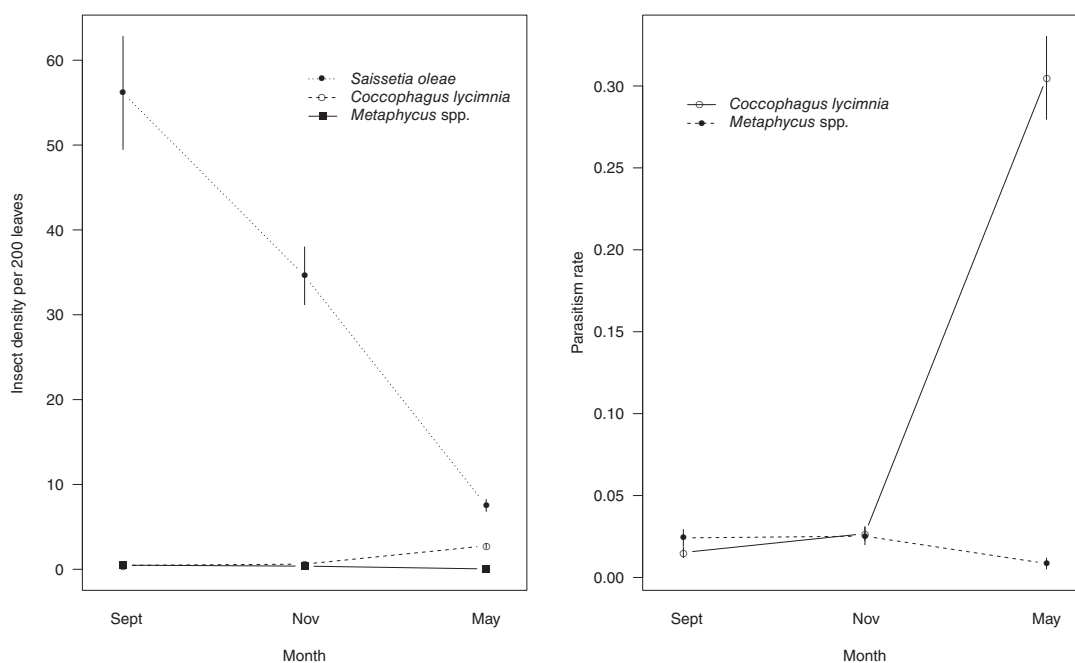
### Sampling protocols

Orchards were sampled in three periods (September and November 2011 and May 2012). These months were selected because the first nymphal parasitoids are often observed in September, and the highest parasitism rates occur from October to November, as well as from April to May (Pereira, 2004). At each sampling period and olive orchard, two twigs (20 cm long) infested with *S. oleae* were collected in 10 randomly selected olive trees. Twigs were isolated in plastic bags and transported in ice chests to the laboratory where they were stored at 6 °C for later observation. Samples were processed within 48 h of collection.

Once in the laboratory, a subsample of 20 leaves per tree was obtained (10 leaves per twig) leading to a total of 200 leaves per orchard. Each subsample was observed under a stereomicroscope to assess the numbers of live and parasitised *S. oleae* specimens of each instar. Parasitised scales were recognised based on their colour as they become yellowish or black depending on whether they are parasitised by *Metaphycus* spp. or *Coccophagus* spp., respectively, as well as by the convex shape of the scale (Pereira, 2004; Tena *et al.*, 2008a; Tena & Garcia-Marí, 2008). To assess host size, the length of the major axis of all second and third nymphal instars (suitable for parasitism by *Metaphycus flavus*, *M. helvolus* and *C. lycimnia*) was measured to the nearest 0.01 mm. Leaves bearing parasitised scales were individually introduced into a glass vial. Vials were sealed with a cotton plug and placed in a climatic chamber at 22 °C, LD 16:8 h and 70% RH. Tubes were checked every 2 days for parasitoid emergence throughout the month following collection. At emergence, parasitoids were identified (Goulet & Hulent, 1993; Guerrieri & Noyes, 2000) and sexed.

### Statistical analyses

In this study several linear models, mixed-effects models, and generalised mixed-effects models were constructed. All analyses were performed in R v.3.4.2 (R Development Team



**Fig. 1.** Seasonal trend of *Saissetia oleae*, its parasitoids *Coccophagus lycimnia* and *Metaphycus* spp., and parasitism by these parasitoids throughout the life cycle of the scale. (a) Number of *S. oleae*, *C. lycimnia* and *Metaphycus* spp.; (b) parasitism rate by *C. lycimnia* and *Metaphycus* spp. Data are means  $\pm$  SE.

2017). Linear models were fitted with the ‘glm’ function in the STATS package, whereas mixed-effects models and generalised mixed-effects models were fitted, respectively, with the ‘lmer’ and ‘glmer’ functions in the LME4 package (Bates *et al.*, 2014). Mixed-effects models were fitted with a Gaussian error distribution, while generalised mixed-effects models had a binomial error distribution with a log-link function. In mixed-effects models, the region at which a given site belonged was included as a random factor to account for the spatial aggregation of sites within a given region. When several insects collected at the same sampling site were used as replicates, site nested within region was included as a random factor to account for both the non-independence of the hosts sampled at the same site, and the spatial aggregation of the sites within a region. In models that had more than one interaction term, non-significant interactions ( $P > 0.05$ ) were removed. In all models, model fit was assessed by visual inspection of the residuals and with a  $\chi^2$  goodness-of-fit test. In linear models the absence of data points with high influence was checked by calculating Cook’s distances. In binomial mixed-effects models, model fit was further assessed with the DHARMA library through a simulation-based approach that creates scaled residuals from fitted generalised linear mixed models with the ‘simulateResiduals’ function in the DHARMA package. The overall uniformity of the residuals was then tested with a Kolmogorov–Smirnov goodness-of-fit test with the ‘testSimulatedResiduals’ function. All binomial mixed-effects models showed no sign of overdispersion, with dispersion factors  $< 1.2$ . Significance of predictor variables (i.e. fixed terms) was usually reported with the ‘summary’ function in R. However, when categorical predictor variables had more

than two different levels (this was the case when testing for seasonal or regional effects), overall significance was reported with likelihood ratio tests using the ANOVA function from the package CAR. This was done to simplify the presentation of results (expressed here as global ANOVA test). *Posthoc* tests in generalised mixed-effects models were performed with the ‘glht’ function in the MULTCOMP package using the Tukey multiple comparison procedure.

The following models were constructed in this study:

- (i) The effect of parasitism on host mortality between months was tested with a mixed-effects model using a Gaussian error distribution with region included as the random factor. The response variable was defined as the difference in the number of live hosts between September and May [expressed as  $\log(\text{mortality} + 0.5)$ ], using as unit of replication the number of hosts in each site. Predictors were host exploitation by all *Metaphycus*, by *C. lycimnia* and their interaction. When exploring the effect of all *Metaphycus* species, a similar model was built but included host exploitation by each species and their interaction with *C. lycimnia* host exploitation.
- (ii) Seasonal and regional patterns of *S. oleae* size and different sized hosts parasitised by *Metaphycus* species and *C. lycimnia* were tested with mixed-effects models using a Gaussian error distribution with site as random factor (region was not included as a random factor but as a fixed predictor). These models included insect size as the response variable and sampling month and region as predictors. In the models for parasitoid host size, the response variable was log-transformed.

- (iii) Partitioning of host resources by parasitoids was tested in a similar manner to a previous study (Pekas *et al.*, 2016). The relationship between the size of available hosts and the size of parasitised hosts by each parasitoid species was analysed by two different methods. In the first, linear models were constructed including mean size of the parasitised hosts at a particular date and site as the response variable, and as predictors mean size of all scales sampled at a particular date and site as continuous, and parasitoid species as categorical. In the second, the Manly selectivity measure (Manly *et al.*, 2002) was also estimated for each parasitoid species. This ratio was obtained by dividing the size of the hosts parasitised by the size of the hosts available at a particular date and site. A *t*-test was used to evaluate whether or not this ratio was different from 1. Significant results expressed how parasitoids used hosts that were bigger or smaller than the average in the environment. Data were not transformed, as the distribution of mean size values was normal. This was checked by plotting quantile–quantile plots.
- (iv) Size-mediated interspecific parasitoid interactions were also tested, in a similar fashion to a previous study using generalised mixed-effects models with a binomial error distribution which included site nested within region as the random factor. As the response variable, each collected host was considered as either parasitised or not; host size and host exploitation by the competing parasitoids (and their interaction term between these variables) were included as predictors. For each parasitoid species and sampling month, a different model was built. In these models, the interaction terms between host size and host exploitation by the competing parasitoid tested our working hypotheses as they expressed whether parasitism on particular host sizes varied with the density of the competing parasitoid.
- (v) Seasonal patterns of *Metaphycus* and *C. lycimnia* secondary sex ratio (emerged adults) were studied with mixed-effects models with a binomial error distribution that included site nested within region as the random factor. These models included the binomial factor that considered each parasitoid to be male or female as the response variable; the parasitoid identity, the sampling month, and their interactions were the predictors.
- (vi) The effect of host size on parasitoid sex ratio was studied with mixed-effects models using a Gaussian error distribution with site nested within region as the random factor. These models included the size of the hosts parasitised [expressed as  $\log(\text{size} + 0.5)$ ] as the response variable; parasitoid species, its sex, and their interaction were the predictors.
- (vii) The effect of size-mediated interspecific parasitoid interactions on secondary sex ratio were studied with mixed-effects models with a binomial error distribution that included site nested within region as the random factor. These models were slightly overdispersed, and thus were corrected by including an observational-level

random factor. For each parasitoid a different model was constructed, and hence the response variable was a binomial factor that considered each individual of a given parasitoid as male or female. The following predictors were included: host exploitation by both the tested parasitoid and the competing species and their pairwise interactions. A significant effect on host exploitation by the tested parasitoid would express density-dependent changes in host exploitation, whereas significant effects on host exploitation by the competing parasitoid would express changes in sex ratio associated to interspecific parasitoid interactions. Significant interaction between host size and host exploitation would indicate the mentioned effects varied with host size.

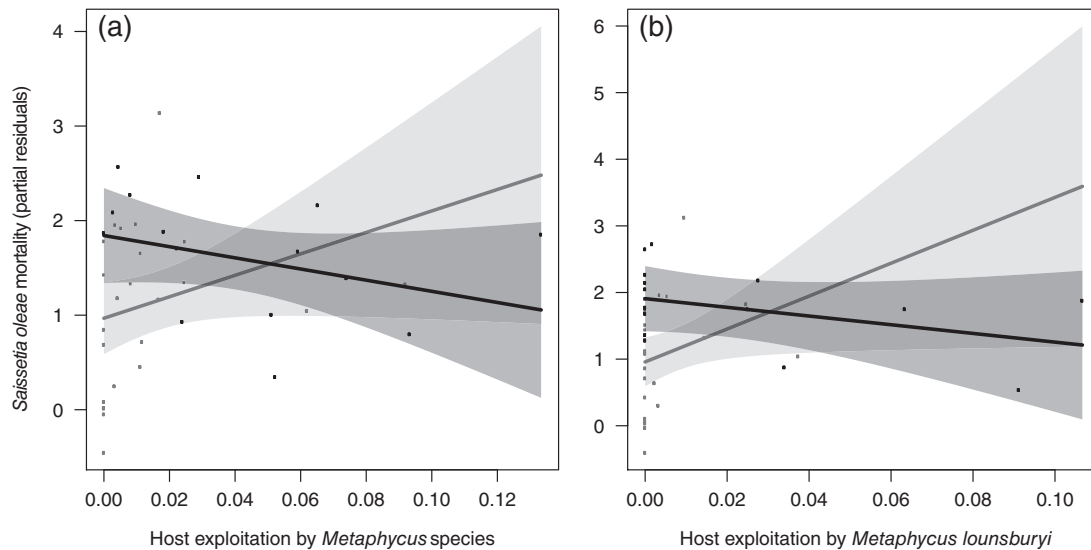
## Results

### *Host abundance, parasitism rates, and interspecific parasitoid interactions*

The first count of *S. oleae* scales was carried out in September, followed by further estimations of density in November and May. Population density decreased with time. The mean number of *S. oleae* scales and nymphs suitable for parasitism (second and third instar) markedly decreased from September to May (Fig. 1). Parasitism by *Metaphycus* species remained low throughout the seasons, whereas *C. lycimnia* parasitism peaked in May (Fig. 1).

The number of hosts that died between September and May was positively related to parasitism rates by *C. lycimnia* in September (mixed-effects model,  $\beta = 28.58$ ,  $t = 2.79$ ,  $n = 33$ ,  $P = 0.0084$ ), but not with parasitism rates by *Metaphycus* ( $\beta = 11.34$ ,  $t = 1.69$ ,  $n = 33$ ,  $P = 0.1$ ). There was, however, a significant interaction between both parasitoid groups ( $\beta = -563.78$ ,  $t = -2.39$ ,  $n = 33$ ,  $P = 0.022$ ); at low *C. lycimnia* parasitism, host mortality increased with *Metaphycus* parasitism in September, whereas at high *C. lycimnia* parasitism, host mortality tended to decrease with *Metaphycus* parasitism (Fig. 2a).

A more detailed model that considered parasitism rates by each of the three *Metaphycus* species individually was also built. After removing non-significant interactions, this model revealed that the number of hosts that died between September and May was positively related to parasitism rates by *C. lycimnia* (mixed-effects model,  $\beta = 31.05$ ,  $t = 3.11$ ,  $n = 31$ ,  $P = 0.0038$ ), but not to those by *M. helvolus* ( $\beta = -25.43$ ,  $t = -1.07$ ,  $n = 31$ ,  $P = 0.2909$ ) or *M. flavus* ( $\beta = 3.36$ ,  $t = 0.39$ ,  $n = 31$ ,  $P = 0.6967$ ). *Metaphycus lounsburyi*, however, had an effect on host mortality that depended on the parasitism of *C. lycimnia*, as revealed by a significant interaction between the rates of parasitism of these two species (*M. lounsburyi* parasitism,  $\beta = 24.69$ ,  $t = 2.02$ ,  $n = 31$ ,  $P = 0.0518$ ; interaction term,  $\beta = -1020.49$ ,  $t = -2.73$ ,  $n = 31$ ,  $P = 0.0106$ ). This interaction shows that at low *C. lycimnia* parasitism, host mortality between September and May increased with *M. lounsburyi* parasitism in September, whereas at high *C. lycimnia* parasitism, host mortality tended to decrease with *M. lounsburyi* parasitism (Fig. 2b).



**Fig. 2.** (a, b) Effect of *Coccophagus lycimnia* and *Metaphycus* spp. (a) or *Metaphycus lounsburyi* parasitism (or host exploitation) (b) on the mortality of *Saissetia oleae* in September. Points represent model partial residuals and fitted lines; 95% confidence intervals are also estimated from the generalised mixed-effects models based on the `visreg` package in R. The interaction represents high (dark grey) and low (light grey) host exploitation values by *C. lycimnia* based on the 50th quantile of this variable.

#### Seasonal and regional patterns of *Saissetia oleae* size and host sizes parasitised by *Metaphycus* and *Coccophagus lycimnia*

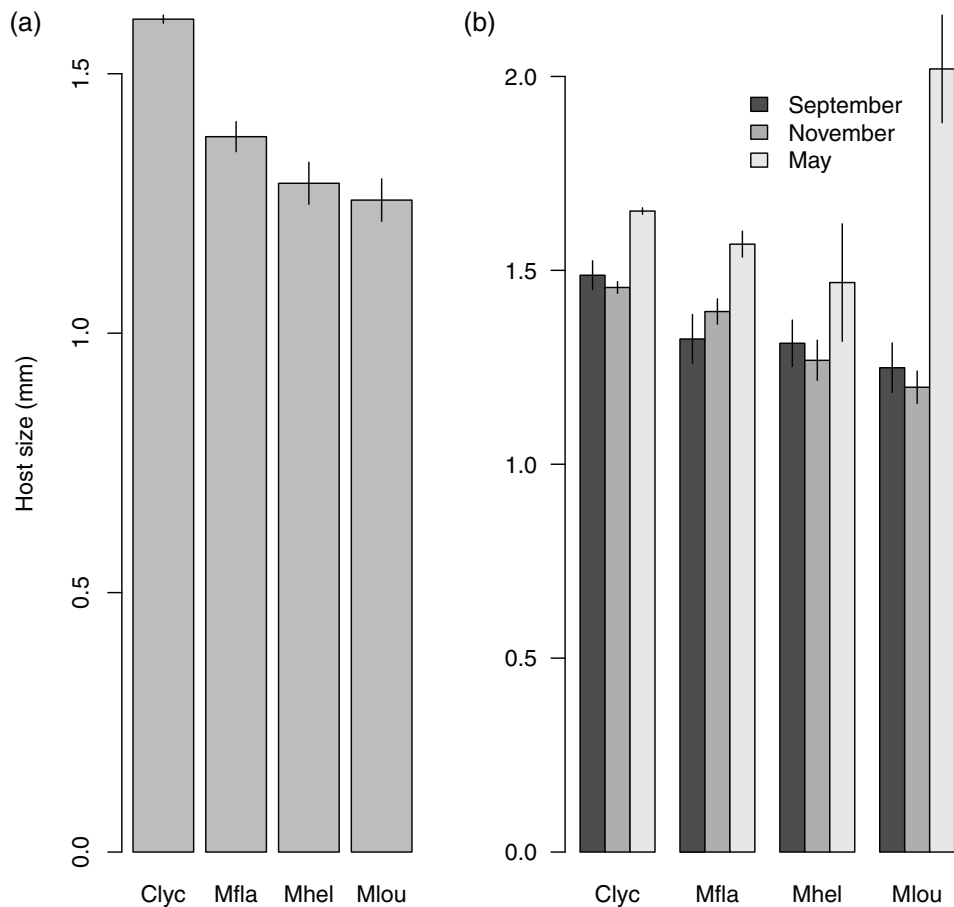
Considering *S. oleae* second and third instars together, mean insect size was significantly different among seasons (global ANOVA mixed-effects model,  $\chi_2^2 = 12.19$ ,  $n = 4287$ ,  $P < 0.0001$ ). Overall, size differences among regions were not significant ( $\chi_5^2 = 3.63$ ,  $n = 4287$ ,  $P = 0.6034$ ), but seasonal differences in scale size varied between regions as revealed by a significant interaction between season and region ( $\chi_{10}^2 = 90.21$ ,  $n = 4287$ ,  $P < 0.0001$ ; Appendix S1; Fig. 2). Overall scale size was  $1.27 \pm 0.01$  mm in September,  $1.22 \pm 0.01$  mm in November, and  $1.57 \pm 0.01$  mm in May; *post hoc* tests revealed scales to be significantly larger in May ( $P < 0.0001$ ).

The hosts from where *C. lycimnia* was reared were significantly larger than those of *Metaphycus* (global ANOVA mixed-effects model for parasitoid genus,  $\chi_1^2 = 51.74$ ,  $n = 1209$ ,  $P < 0.0001$ ). Differences were also significant among the three seasons: September, November and May (effect for season,  $\chi_2^2 = 116.12$ ,  $n = 1209$ ,  $P < 0.0001$ ). The seasonal effect was consistent between the two parasitoid genera as revealed by a non-significant interaction between these two fixed terms (interaction effect,  $\chi_2^2 = 1.47$ ,  $n = 1209$ ,  $P = 0.48$ ). When the *Metaphycus* species are considered separately, the size of the hosts from where the four parasitoids were reared differed significantly (mixed-effects model for parasitoid species,  $\chi_3^2 = 53.19$ ,  $n = 1151$ ,  $P < 0.0001$ ). Host size used by the four parasitoids also varied among seasons (mixed-effects model for season,  $\chi_2^2 = 121.42$ ,  $n = 1151$ ,  $P < 0.0001$ ), and the seasonal effect varied depending on the parasitoid species as revealed by a significant interaction ( $\chi_6^2 = 19.42$ ,  $n = 1151$ ,  $P = 0.0035$ ; Fig. 3). Within this mixed-effects model, *post hoc* tests reveal that *C. lycimnia* hosts were significantly larger than hosts of *M.*

*flavus* and *M. lounsburyi* ( $P < 0.05$ ); however, they were not significantly different from *M. helvolus* hosts ( $P > 0.05$ ). Among seasons, parasitised scales in May were significantly larger than those parasitised in September or November ( $P < 0.0001$ ); scale size was not significantly different between September and November ( $P = 0.4445$ ).

#### Partitioning of host resources by parasitoids

Considering the mean for each sampling date and study site, the size of parasitised scales by *C. lycimnia* and *Metaphycus* was significantly correlated with the mean size of hosts available in the scale population (linear model host size effect,  $\beta = 0.65$ ,  $t = 5.55$ ,  $n = 148$ ,  $P < 0.0001$ ) (Fig. 4a). In this model, parasitoid genus was also a significant explanatory variable. Hosts from where *C. lycimnia* was reared were larger than those of *Metaphycus* (linear model parasitoid effect with the *Metaphycus* genus as reference category,  $\beta = 0.39$ ,  $t = 2.04$ ,  $n = 148$ ,  $P = 0.043$ ). There was not a significant interaction between parasitoid species and host size, and hence the slope of the relationship between the size of the hosts available and those used did not depend on the parasitoid genus ( $\beta = -0.16$ ,  $t = -1.13$ ,  $n = 148$ ,  $P = 0.26$ ). If the different *Metaphycus* species are considered for each sampling date and study site, the mean size of scales parasitised by *C. lycimnia*, *M. flavus*, *M. helvolus* and *M. lounsburyi* was significantly correlated with the mean size of the hosts available in the population (linear model size effect,  $\beta = 0.48$ ,  $t = 5.41$ ,  $n = 168$ ,  $P < 0.0001$ ) (Fig. 4b). Relative to *C. lycimnia* (used in this model as the reference category), only *M. lounsburyi* used smaller hosts (linear model parasitoid effect for *M. flavus*,  $\beta = -0.10$ ,  $t = -0.42$ ,  $n = 168$ ,  $P = 0.68$ ; for *M. helvolus*,  $\beta = -0.08$ ,  $t = -0.28$ ,  $n = 168$ ,  $P = 0.79$ ; for *M. lounsburyi*,  $\beta = -1.17$ ,  $t = -4.23$ ,  $n = 168$ ,



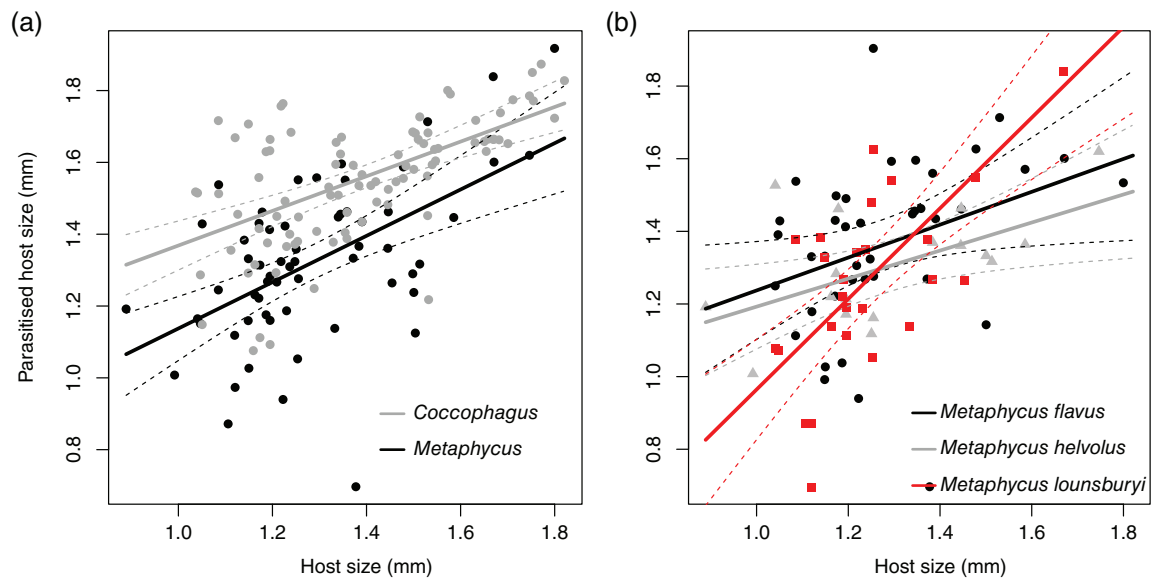
**Fig. 3.** (a, b) Size of *Saissetia oleae* (host size) (a) from which the parasitoids *Coccophagus lycimnia* (Clyc) and several species of *Metaphycus* [*M. flavus* (Mfla), *M. helvolus* (Mhel) and *M. lounsburyi* (Mlou)] emerged in different seasons (b).

$P < 0.0001$ ). Moreover, *M. lounsburyi* had a different host exploitation strategy from that of the other parasitoids. At large host sizes, it parasitised larger hosts than the other three parasitoids, whereas at smaller host sizes it parasitised smaller hosts (linear model interaction effect between host size and parasitism by *M. flavus*,  $\beta = -0.03$ ,  $t = -0.16$ ,  $n = 168$ ,  $P = 0.87$ ; by *M. helvolus*,  $\beta = -0.10$ ,  $t = -0.45$ ,  $n = 168$ ,  $P = 0.65$ ; by *M. lounsburyi*,  $\beta = 0.76$ ,  $t = 3.59$ ,  $n = 168$ ,  $P = 0.0004$ ) (Fig. 4b). The Manly selectivity measure revealed that both *C. lycimnia* and *M. flavus*, but not *M. helvolus* and *M. lounsburyi*, used hosts larger than the average found in the host population. Manly's ratio was significantly larger than the one in *C. lycimnia* ( $1.13 \pm 0.02$ ,  $t_{91} = 8.05$ ,  $P < 0.0001$ ) and *M. flavus* ( $1.08 \pm 0.03$ ,  $t_{39} = 2.55$ ,  $P = 0.015$ ), but did not differ from that in *M. helvolus* ( $1.03 \pm 0.04$ ,  $t_{16} = 0.73$ ,  $P = 0.47$ ) and *M. lounsburyi* ( $1.02 \pm 0.03$ ,  $t_{26} = 0.61$ ,  $P = 0.54$ ).

#### Size-mediated interspecific parasitoid interactions

The probability of parasitism was explored with mixed-effect models using a binomial error distribution which considered each host as either parasitised or not. When considering all

*Metaphycus* species together, *C. lycimnia* parasitism was positively related to host size in September, November and May, and positively related to *Metaphycus* host exploitation in May, but not in September and November (Table 1). These effects were consistent throughout *Metaphycus* host exploitation intensities in September and November, but not in May. In May, *C. lycimnia* parasitism was positively related to host size, but the slope of the correlation was lower at large host exploitation by *Metaphycus*, as revealed by a significant interaction between host size and host exploitation by *Metaphycus*. Thus host exploitation by *Metaphycus* had a negative effect on *C. lycimnia* parasitism through competition for larger hosts (Table 1; Fig. 5a). Parasitism by *Metaphycus* was related to host size in November only, and the interaction terms between host size and parasitism by *C. lycimnia* were never significant in these models (Table 1). Similar results were obtained when the *Metaphycus* species were analysed separately. Parasitism by *C. lycimnia* was always positively related to host size in the three sampling months when including host exploitation by any of the three *Metaphycus* species (Table 2). In the models that include host exploitation by *M. flavus*, none of the other variables were significant in any of the 3 months studied. In the models including host exploitation by *M. helvolus*, however, host exploitation by this



**Fig. 4.** (a, b) Relationship between the size of the hosts available and the size of the hosts attacked by *Coccophagus lycimnia* (light grey) and *Metaphycus* spp. (black) (a) and each *Metaphycus* species [*M. flavus* (black), *M. helvolus* (light grey) and *M. lounsburyi* (red)] (b) at each sampling date and orchard. Lines represent independent linear regression models with 95% confidence intervals for each parasitoid.

**Table 1.** Effect of host size and host exploitation by the competing parasitoid on parasitism by the facultative autoparasitoid *Coccophagus lycimnia* and *Metaphycus* spp. on the soft scale *Saissetia oleae*.

Fixed factors	September ( <i>n</i> = 1323)			November ( <i>n</i> = 1585)			May ( <i>n</i> = 1284)		
	$\beta$	<i>z</i>	<i>P</i>	$\beta$	<i>z</i>	<i>P</i>	$\beta$	<i>z</i>	<i>P</i>
Parasitism by <i>C. lycimnia</i>									
Intercept	-6.8	-6.278	< 0.0001	-6.28	-10.131	< 0.001	-3.59	-7.431	< 0.0001
Host size	<b>2.05</b>	<b>2.875</b>	<b>0.0041</b>	<b>3.12</b>	<b>7.633</b>	<b>&lt; 0.001</b>	<b>2.53</b>	<b>9.446</b>	<b>&lt; 0.0001</b>
<i>Metaphycus</i> host exploitation	1.86	0.086	0.9318	-16.21	-0.926	0.3552	<b>44.07</b>	<b>2.538</b>	<b>0.0111</b>
Host size $\times$ <i>Metaphycus</i> host exploitation	8.21	0.555	0.5787	5.96	0.501	0.6179	<b>-28.22</b>	<b>-3.168</b>	<b>0.0015</b>
Parasitism by <i>Metaphycus</i>									
Intercept	-4.3	-4.089	< 0.0001	-4.37	-6.387	< 0.001	-0.74	-0.272	0.7853
Host size	0.01	0.021	0.9832	<b>1.01</b>	<b>2.159</b>	<b>0.0309</b>	-2.75	-1.511	0.1311
<i>C. lycimnia</i> host exploitation	0.81	0.028	0.9772	2.35	0.157	0.8751	-15.37	-1.932	0.0534
Host size $\times$ <i>C. lycimnia</i> host exploitation	9.07	0.447	0.6553	1.57	0.141	0.8879	8.17	1.677	0.0936

Cases of significant differences are shown in bold. The significant interactions are represented graphically in Fig. 3.

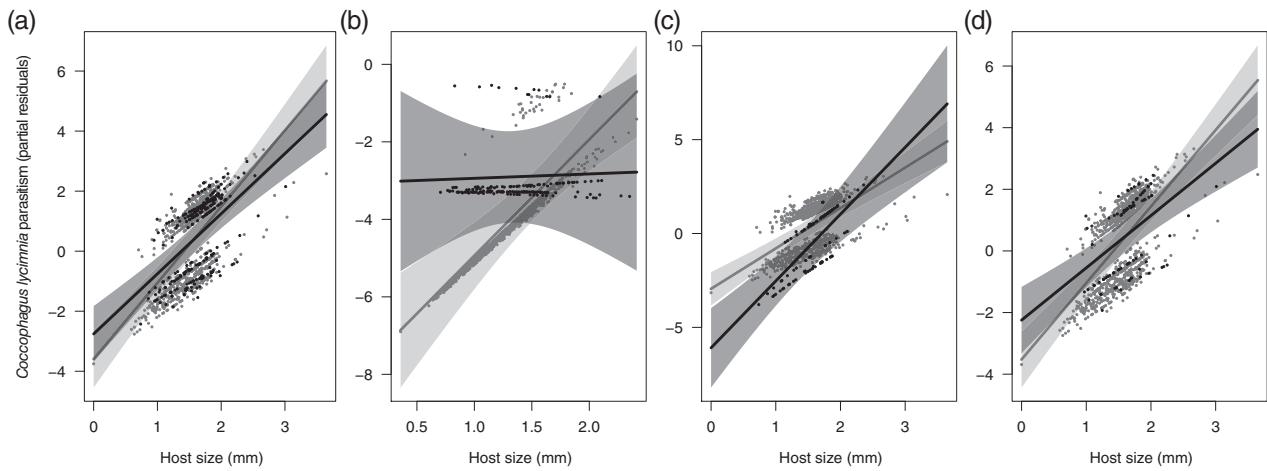
parasitoid in September and May had a significant effect on *C. lycimnia* parasitism. This effect depended on host size but in different directions depending on the month. In September, the slope of the positive relationship between *C. lycimnia* parasitism and host size was smaller with large host exploitation by *M. helvolus* (Table 1; Fig. 5b), whereas the opposite was true in May (Table 1; Fig. 5c). In May, when the parasitoid *M. lounsburyi* had large host exploitation values, the slope of the positive correlation between *C. lycimnia* parasitism and host size was also smaller (Table 1; Fig. 5d).

#### Seasonal patterns of *Metaphycus* and *Coccophagus lycimnia* secondary sex ratio and effect of host size

The secondary sex ratio of *C. lycimnia* and of the different *Metaphycus* species was female-biased, particularly for

*C. lycimnia*: 92% of females in *C. lycimnia*, 60% in *M. flavus*, 55% in *M. helvolus* and 82% in *M. lounsburyi*. The proportion of females varied significantly between *C. lycimnia* and *Metaphycus* (global ANOVA mixed-effects model for parasitoid genus,  $\chi_1^2 = 33.06$ , *n* = 1285, *P* < 0.0001) and between months (effect for season,  $\chi_2^2 = 48.39$ , *n* = 1285, *P* < 0.0001); these differences also depended on the parasitoid genus (interaction effect,  $\chi_2^2 = 18.25$ , *n* = 1285, *P* = 0.0001). A mixed-effects model that included all *Metaphycus* species revealed that both parasitoid species (global ANOVA mixed-effects model for parasitoid species,  $\chi_1^2 = 54.45$ , *n* = 1285, *P* < 0.0001) and sampling month (effect for season,  $\chi_2^2 = 46.67$ , *n* = 1285, *P* < 0.0001) had a significant effect on secondary sex ratio, while the seasonal effect depended on the parasitoid species (interaction effect,  $\chi_2^2 = 22.51$ , *n* = 1285, *P* = 0.0009). In simpler models limited to each parasitoid species, the proportion of *C. lycimnia* females



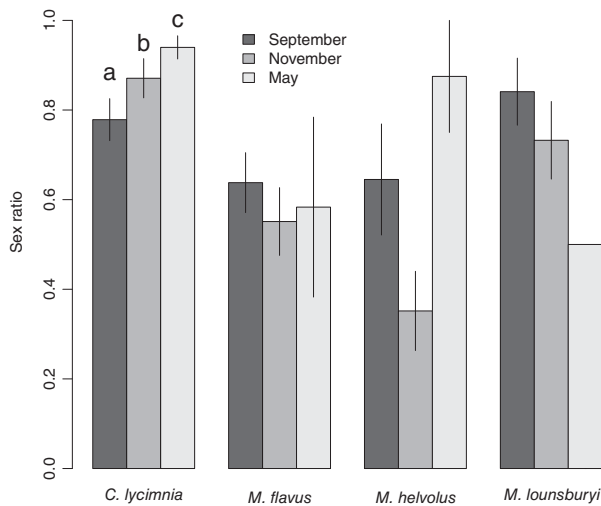


**Fig. 5.** Effect of *Saissetia oleae* size (host size) on *Coccophagus lycimnia* parasitism and its interaction with host exploitation by different *Metaphycus* species: (a) *Metaphycus* spp. in May; (b) *M. helvolus* in September; (c) *M. helvolus* in May; and (d) *M. lounsburyi* in May. Points represent model partial residuals and fitted lines; 95% confidence intervals are also estimated from the generalised mixed-effects models based on the *VISREG* package in R. The interaction represents high (dark grey) and low (light grey) host exploitation values by *Metaphycus* based on the 50th quantile of this variable.

**Table 2.** Effect of host size and host exploitation by the competing parasitoid on parasitism by the facultative autoparasitoid *Coccophagus lycimnia* and three species of the genus *Metaphycus* on the soft scale *Saissetia oleae*.

Fixed factors	September ( <i>n</i> = 1323)			November ( <i>n</i> = 1585)			May ( <i>n</i> = 1284)		
	$\beta$	<i>z</i>	<i>P</i>	$\beta$	<i>z</i>	<i>P</i>	$\beta$	<i>z</i>	<i>P</i>
<b>Parasitism by <i>C. lycimnia</i></b>									
Intercept	-6.98	-7.154	<0.0001	-6.52	-10.651	<0.0001	-3.30	-7.093	<0.0001
Host size	<b>2.29</b>	<b>3.631</b>	<b>0.0002</b>	<b>3.24</b>	<b>8.080</b>	<b>&lt;0.0001</b>	<b>2.32</b>	<b>8.962</b>	<b>&lt;0.0001</b>
<i>M. flavus</i> host exploitation	9.65	0.239	0.8107	-14.56	-0.409	0.6830	45.91	0.993	0.3210
Host size $\times$ <i>M. flavus</i> host exploitation	3.15	0.117	0.9066	2.44	0.102	0.9190	-26.01	-1.014	0.3110
Intercept	-7.93	-8.774	<0.0001	-6.71	-12.110	<0.0001	-2.95	-6.470	<0.0001
Host size	<b>2.99</b>	<b>5.353</b>	<b>&lt;0.0001</b>	<b>3.46</b>	<b>9.534</b>	<b>&lt;0.0001</b>	<b>2.15</b>	<b>8.607</b>	<b>&lt;0.0001</b>
<i>M. helvolus</i> host exploitation	<b>256.61</b>	<b>3.345</b>	<b>0.0008</b>	12.82	0.247	0.8050	<b>-165.59</b>	<b>-2.764</b>	<b>0.0057</b>
Host size $\times$ <i>M. helvolus</i> host exploitation	<b>-151.66</b>	<b>-2.863</b>	<b>0.0042</b>	-34.45	-1.016	0.3090	<b>74.16</b>	<b>2.090</b>	<b>0.0367</b>
Intercept	-6.49	-6.597	<0.0001	-6.23	-11.506	<0.0001	-3.52	-7.576	<0.0001
Host size	<b>1.91</b>	<b>3.169</b>	<b>0.0015</b>	<b>2.96</b>	<b>8.551</b>	<b>&lt;0.0001</b>	<b>2.48</b>	<b>9.591</b>	<b>&lt;0.0001</b>
<i>M. lounsburyi</i> host exploitation	-26.22	-0.831	0.4059	-62.77	-1.811	0.0701	<b>67.18</b>	<b>3.011</b>	<b>0.0026</b>
Host size $\times$ <i>M. lounsburyi</i> host exploitation	30.50	1.432	0.1522	44.33	1.898	0.0576	<b>-41.32</b>	<b>-3.583</b>	<b>0.0003</b>
<b>Parasitism by <i>M. flavus</i></b>									
Intercept	-5.04	-3.606	0.0003	-6.45	-6.160	<0.0001	-6.92	-0.527	0.5980
Host size	0.38	0.371	0.7103	<b>1.97</b>	<b>2.764</b>	<b>0.0057</b>	-1.96	-0.224	0.8230
<i>C. lycimnia</i> host exploitation	15.70	0.381	0.7029	7.02	0.276	0.7828	2.75	0.110	0.9120
Host size $\times$ <i>C. lycimnia</i> host exploitation	2.51	0.086	0.9311	-1.83	-0.101	0.9193	3.30	0.198	0.8430
<b>Parasitism by <i>M. helvolus</i></b>									
Intercept	-19.98	-2.354	0.0185	-4.80	-3.585	0.0003	-1.64	-0.369	0.7120
Host size	5.49	1.279	0.2008	-0.07	-0.071	0.9434	-1.57	-0.512	0.6090
<i>C. lycimnia</i> host exploitation	207.18	1.345	0.1787	-24.64	-0.689	0.4909	-21.81	-0.836	0.4030
Host size $\times$ <i>C. lycimnia</i> host exploitation	-102.57	-0.990	0.3221	11.80	0.456	0.6487	6.48	0.406	0.6850
<b>Parasitism by <i>M. lounsburyi</i></b>									
Intercept	-6.35	-1.527	0.1271	-6.18	-3.688	0.0002	-14.76	-1.041	0.2980
Host size	-0.86	-0.839	0.4010	0.20	0.260	0.7948	1.68	0.240	0.8100
<i>C. lycimnia</i> host exploitation	-51.31	-1.071	0.2850	7.07	0.305	0.7602	1.32	0.042	0.9660
Host size $\times$ <i>C. lycimnia</i> host exploitation	31.11	0.963	0.3360	-3.26	-0.171	0.8645	1.14	0.073	0.9420

Cases of significant differences are shown in bold. The significant interactions are represented graphically in Fig. 5.



**Fig. 6.** Sex ratio (mean  $\pm$  SE) and host size of the different parasitoids studied: the autoparasitoid *Coccophagus lycimnia* and the primary parasitoids *Metaphycus flavus*, *Metaphycus helvolus* and *Metaphycus lounsburyi* on different seasons. Different letters denote significant differences among seasons. *P*-values were considered significant after Bonferroni correction ( $P < 0.05$ ).

was larger in May than in September (*post hoc* tests in Fig. 6), whereas these differences were not significant for *M. flavus*, *M. helvolus* and *M. lounsburyi* ( $P > 0.2$ ; Fig. 6).

When considering all *Metaphycus* species together, the size of the hosts from where *C. lycimnia* emerged were significantly larger than those from where *Metaphycus* emerged (mixed-effects model for parasitoid species,  $\beta = -0.15$ ,  $t = -12.03$ ,  $n = 1146$ ,  $P < 0.0001$ ). In addition, females of *C. lycimnia*, but not of *Metaphycus*, emerged from larger hosts than did males (mixed-effects model for parasitoid species,  $\beta = -0.05$ ,  $t = -3.25$ ,  $n = 1146$ ,  $P = 0.0012$ ; interaction effect between parasitoid species and secondary sex ratio,  $\beta = 0.05$ ,  $t = 2.16$ ,  $n = 1146$ ,  $P = 0.031$ ). Results were slightly different when the different *Metaphycus* species were

considered. As already shown in the previous analysis, *M. flavus*, *M. helvolus* and *M. lounsburyi* used smaller hosts than *C. lycimnia* (mixed-effects model for parasitoid species: *M. flavus*,  $\beta = -0.11$ ,  $t = -5.76$ ,  $n = 1146$ ,  $P < 0.0001$ ; *M. helvolus*,  $\beta = -0.14$ ,  $t = -5.21$ ,  $n = 1146$ ,  $P < 0.0001$ ; *M. lounsburyi*,  $\beta = -0.19$ ,  $t = -10.37$ ,  $n = 1146$ ,  $P < 0.0001$ ); males also emerged from smaller hosts ( $\beta = -0.06$ ,  $t = -3.93$ ,  $n = 1146$ ,  $P < 0.0001$ ) (Appendix S1; Fig. 3). The interaction between secondary sex ratio and parasitoid species, however, was only significant for *M. lounsburyi*; in this species, females emerged from smaller hosts than did males. This interaction was not evident for either *M. flavus* or *M. helvolus* (interaction effect between secondary sex ratio and *M. flavus*,  $\beta = 0.04$ ,  $t = 1.24$ ,  $n = 1146$ ,  $P = 0.2150$ ; *M. helvolus*,  $\beta = 0.03$ ,  $t = 0.63$ ,  $n = 1146$ ,  $P = 0.5285$ ; *M. lounsburyi*,  $\beta = 2.51$ ,  $t = -10.37$ ,  $n = 1146$ ,  $P = 0.0121$ ).

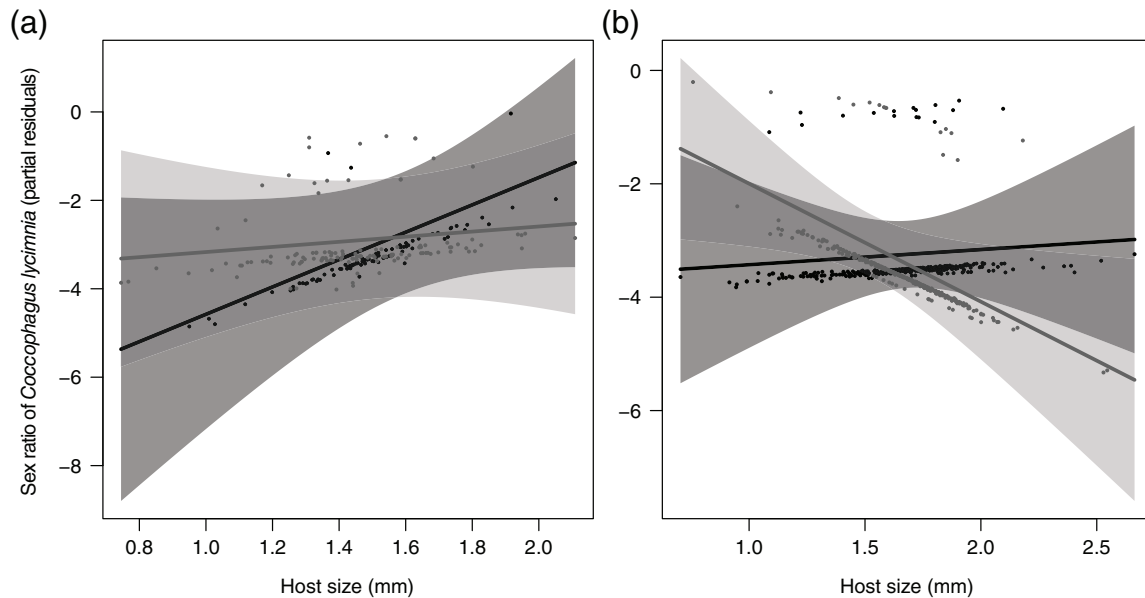
#### Effect of size-mediated interspecific parasitoid interactions on secondary sex ratio

The probability of producing a female was explored with mixed-effect models using a binomial error distribution that considered each parasitoid as either female or male. For each sampling period, a different model was constructed to test specific hypotheses based on the included fixed factors and interactions among them. These analyses revealed that in September none of the variables included had a significant effect on female production for any parasitoid (Table 3). In November, the density of *Metaphycus* parasitoids had a positive effect on the production of *C. lycimnia* females. This result suggests that *C. lycimnia* may have a secondary sex ratio that is mediated by antagonistic interactions. In addition, this response depended on host size as revealed by a significant interaction between host size and *Metaphycus* host exploitation. The proportion of *C. lycimnia* females was positively related to host size but only at low levels of host exploitation by *Metaphycus* (Table 3; Fig. 7a). In May, at low *C. lycimnia* densities, the relationship between host size and secondary sex ratio was not evident; however,

**Table 3.** Effect of host size and host exploitation on the secondary sex ratio of the facultative autoparasitoid *Coccophagus lycimnia* and *Metaphycus* spp. when they parasitise the soft scale *Saissetia oleae*.

Fixed factors	September ( $n = 1323$ )			November ( $n = 1585$ )			May ( $n = 1284$ )		
	$\beta$	$z$	<i>P</i>	$\beta$	$z$	<i>P</i>	$\beta$	$z$	<i>P</i>
<i>C. lycimnia</i> probability of female									
Intercept	5.42	1.377	0.1690	-8.70	-2.456	0.0140	-7.21	-2.242	0.0249
Host size	-5.11	-1.563	0.1180	3.85	1.765	0.0775	2.45	1.292	0.1964
<i>Metaphycus</i> host exploitation	2.87	0.246	0.8060	<b>221.21</b>	<b>2.072</b>	<b>0.0383</b>	10.37	1.421	0.1553
<i>C. lycimnia</i> host exploitation	24.88	1.155	0.2480	-3.36	-0.224	0.8231	<b>14.10</b>	<b>1.966</b>	<b>0.0493</b>
Size $\times$ <i>Metaphycus</i> host exploitation				<b>-141.84</b>	<b>-1.979</b>	<b>0.0478</b>			
Size $\times$ <i>C. lycimnia</i> host exploitation							<b>-8.77</b>	<b>-2.001</b>	<b>0.0454</b>
<i>Metaphycus</i> probability of female									
Intercept	0.44	0.237	0.8130	-1.44	-1.278	0.2010	NA	NA	NA
Host size	-0.99	-0.654	0.5130	0.50	0.639	0.5230	NA	NA	NA
<i>Metaphycus</i> host exploitation	-8.49	-0.809	0.4190	3.12	0.818	0.4130	NA	NA	NA
<i>C. lycimnia</i> host exploitation	0.45	0.023	0.9820	2.47	0.368	0.7130	NA	NA	NA

Cases of significant differences are shown in bold. The significant interactions are represented graphically in Fig. 7.



**Fig. 7.** (a, b) Effect of the interaction between *Metaphycus* spp. parasitism and *Saissetia oleae* size on the secondary sex ratio of *Coccophagus lycimnia* in November (a) and May (b). The fitted lines were estimated from the generalised mixed-effects models, and the points represent the partial residuals of the models based on the *visreg* package in R. The interaction represents high (dark grey) and low (light grey) host exploitation values by *Metaphycus* based on the 50th quantile of this variable.

this relationship was negative at high *C. lycimnia* densities. As *C. lycimnia* males can be produced via facultative hyperparasitism of its own females (autoparasitism), it is likely that high *C. lycimnia* densities promote this behaviour; hence the number of males in large hosts was increased (Fig. 7b). The production of *Metaphycus* females was not altered by either host size or antagonistic interactions. Due to a low number of insects obtained, however, we were not able to test these effects in May (Table 3).

## Discussion

Our study reports a case of competition between primary parasitoids and an autoparasitoid attacking a common host, along with the effect of this competition on host suppression under field conditions. Our results suggest that an autoparasitoid, although an inferior exploitative competitor (i.e. it attacks common hosts later in their development than the primary parasitoid), can outcompete the primary parasitoid without disrupting the suppression of their common host. Even though theoretical studies suggest that this should be the case in most scenarios, there is currently little evidence of this outcome, particularly under field conditions.

*Coccophagus lycimnia* outcompeted parasitoids of the genus *Metaphycus* when parasitizing *S. oleae* nymphs. Our analyses were primarily designed to detect the mechanism and effects of competition on host exploitation by both parasitoid genera and the density of their common host. Although parasitism rates were very low at the beginning of the scale life cycle (September), our data suggest that scale mortality is positively related to the parasitism rates of *C. lycimnia* during this season, while

independent of *Metaphycus* parasitism during the same season. Therefore, *C. lycimnia* reduced the population of soft scales independently of the primary parasitoids of the genus *Metaphycus*. Later on, in May, parasitism rates by the autoparasitoid reached almost 30%. Theory predicts that pest suppression will be disrupted except when primary parasitoids and autoparasitoids are equally effective at suppressing the pest (Briggs & Collier, 2001). Our results are in disagreement with this prediction because, although *C. lycimnia* uses hosts later in their development (i.e. of larger size) than do parasitoids of the *Metaphycus* genus, we found the autoparasitoid to be more efficient than the primary parasitoids in suppressing *S. oleae* in northern Portugal olive trees.

Two previous studies addressed the effects of competition between primary and autoparasitoids on the population of a common host, though in field cages (Bográn *et al.*, 2002; Hunter *et al.*, 2002). Our work differs with previous studies in the literature in that natural populations in their natural environment were used. The two previous studies are based on the use of caged plants and the subsequent release of parasitoids. This methodology allowed host population densities to be compared when parasitoids were absent, released alone, or in competition, so that their effect could be determined. However, this inoculative methodology did not consider the dynamic phenology of the distinct species (i.e. different arrival times within the season, so mixtures of host and parasitoid ages, etc.) as well as their dispersal from other patches (i.e. metapopulation dynamics). The arrival and attack of the distinct species might differ according to the phenology of the host and their climatic necessities, as well as other factors such as alternative hosts, surrounding vegetation, margins, etc. (Snyder *et al.*, 2005). In fact, metapopulation dynamics may account

for the persistence and success of biological control in systems (Murdoch *et al.*, 1996). In our study, three different *Metaphycus* species were found, and they differed in their host exploitation strategies (particularly *M. lounsburyi*). This result emphasises the importance of analysing changes in the metacommunity when exploring the success or failure of biological control programmes.

*Metaphycus* species are usually considered superior competitors and more effective biological control agents than *C. lycimnia* because they can outcompete the autoparasitoid through exploitative competition using hosts of smaller size (Bernal *et al.*, 2001; Kapranas *et al.*, 2007; Tena & Garcia-Marí, 2008; Kapranas & Tena, 2015; herein). The exploitation of smaller hosts by parasitoids of whiteflies and scale insects (including armoured scales, soft scales and mealybugs) is a widely documented mechanism that explains the superiority of the primary parasitoids and sometimes the displacement of competitor parasitoids that need larger hosts to develop (Luck & Podoler, 1985). In our study, however, we found the opposite, as *C. lycimnia* was the stronger competitor, with clearer impacts on pest suppression compared with the primary parasitoids. *Metaphycus flavus* and *M. helvolus* used smaller hosts than *C. lycimnia*; however, this preemptive resource exploitation did not permit them to outcompete *C. lycimnia*.

Size-mediated antagonistic interactions can allow competing species to coexist. Recently, we demonstrated this with the primary parasitoids *Aphytis melinus* DeBach and *Aphytis chrysomphali* (Hymenoptera: Aphelinidae) (Pekas *et al.*, 2016). These parasitoids can coexist when they parasitise the armoured scale *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae), because the weaker competitor (*A. chrysomphali*) survives on smaller hosts when the density of the superior competitor (*A. melinus*) is high. This host exploitation pattern provides the poorer competitor with an competition-free space. In our study, however, we could not observe this coexistence mechanism because the poorer competitor, *Metaphycus*, did not use smaller hosts when *C. lycimnia* parasitism was high. This was revealed by non-significant interactions between host size and host exploitation by *C. lycimnia* on the parasitism rates of *Metaphycus*. Similarly, Bográn *et al.* (2002) found the primary parasitoid *E. mundus* to use smaller hosts in the presence of the autoparasitoid *E. pergandiella* than when released alone. On the other hand, the significant interaction between host size and host exploitation by *Metaphycus* on parasitism by *C. lycimnia* suggests that the autoparasitoid might change its host exploitation pattern depending on *Metaphycus* density. This potential change in host exploitation might also explain why *Metaphycus* parasitism did not recover later in the life cycle of its host.

Our results also provide insights into other potential mechanisms and potential life-history strategies that allow *C. lycimnia* to outcompete *Metaphycus* parasitoids. First, *C. lycimnia* had a higher proportion of females than *Metaphycus* in the three studied seasons. In addition, the secondary sex ratio varied with antagonistic interactions, as the proportion of females did not increase with host size when *Metaphycus* host exploitation increased in November. As *C. lycimnia* is a facultative autoparasitoid, this result suggests that *C. lycimnia* might prefer to use *Metaphycus* females rather than their own females as a

secondary host to produce males. Such host preference would provide *C. lycimnia* with a substantial competitive advantage over *Metaphycus* parasitoids, which may partially explain why the mortality of the host throughout the study was independent of *Metaphycus* parasitism when *C. lycimnia* levels were high. Zang *et al.* (2011) found that *Encarsia sophia*, an autoparasitoid of whiteflies, also prefers to use heterospecific hosts as secondary hosts for producing males in a choice test in the laboratory. This preference could be due to the higher fitness of males developed on heterospecific females as compared with those developed on conspecific ones (Zang *et al.*, 2011). Bernal *et al.* (2001) also encountered a positive relationship between the numbers of *Metaphycus* and the proportion of *C. lycimnia* males in field conditions when parasitizing the soft scale, *C. pseudomagnoliarum*. Nevertheless, the authors suggested that the high population densities of *C. lycimnia* might reduce the density of *Metaphycus* species and explain the negligible control of citricola scale in California's San Joaquin Valley (Bernal *et al.*, 2001).

## Conclusions

Herbivore suppression may depend on intricate interactions among multiple parasitoid species that have important implications for pest regulation. This is particularly true for species with uncommon life-history strategies, such as autoparasitoids. In the case of soft scales, such as *S. oleae*, they are generally controlled by a complex of primary parasitoids of the family Encyrtidae, which are attacked by autoparasitoids of genus *Coccophagus* (Kapranas & Tena, 2015). As *Coccophagus* spp. can negatively influence the population dynamics of their encyrtid hosts, their use in biological control programmes has been limited. However, our data show that *C. lycimnia*, an autoparasitoid that is inferior at resource exploitation, outcompeted the complex of primary parasitoids of the genus *Metaphycus* and regulated the population of their common host, *S. oleae*, in olive trees from Portugal. Therefore, the role of *C. lycimnia* as a biological control agent of soft scales should be reconsidered in future biological control programmes. Finally, our study will also contribute to understanding how autoparasitoids may outcompete primary parasitoids and affect the outcome of biological control efforts in agriculture.

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## Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Appendix S1.** *Saissetia oleae* size and its effect on parasitoid exploitation and sex ratio.

## References

- Bates, D., Maechler, M., Bolker, B. & Walker, S. (2014) *lme4: Linear mixed-effects models using Eigen and S4*. R package, version 1.1-7. <http://CRAN.R-project.org/package=lme4>.
- Ben-Dov, Y. & Hodgson, C.J. (1997) *Soft Scale Insects: Their Biology, Natural Enemies and Control*. Elsevier Science B.V., Amsterdam, the Netherlands.
- Bernal, J.S., Luck, R.F. & Morse, J.G. (1998) Sex ratios in field populations of two parasitoids (hymenoptera: Chalcidoidea) of *Coccus hesperidum* L. (Homoptera: Coccidae). *Oecologia*, **116**, 510–518.
- Bernal, J.S., Luck, R.F. & Morse, J.G. (1999) Augmentative release trials with *Metaphycus* spp. (hymenoptera: Encyrtidae) against citricola scale (Homoptera: Coccidae) in California's San Joaquin Valley. *Journal of Economic Entomology*, **92**, 1099–1107. <https://doi.org/10.1093/jee/92.5.1099>.
- Bernal, J.S., Luck, R.F., Morse, J.G. & Drury, M.S. (2001) Seasonal and scale size relationships between citricola scale (Homoptera: Coccidae) and its parasitoid complex (hymenoptera: Chalcidoidea) on San Joaquin Valley citrus. *Biological Control*, **20**, 210–221.
- Bográn, C.E., Heinz, K.M. & Ciomperlik, M.A. (2002) Interspecific competition among insect parasitoids: field experiments with whiteflies as hosts in cotton. *Ecology*, **83**, 653–668.
- Boivin, G. & Brodeur, J. (2006) *Intra- and Interspecific Interactions Among Parasitoids: Mechanisms, Outcomes and Biological Control. Trophic and Guild Interactions in Biological Control* (pp. 123–144). Springer, Dordrecht, the Netherlands.
- Borer, E.T., Briggs, C.J., Murdoch, W.W. & Swarbrick, S.L. (2003) Testing intraguild predation theory in a field system: does numerical dominance shift along a gradient of productivity? *Ecology Letters*, **6**, 929–935.
- Borer, E.T., Murdoch, W.W. & Swarbrick, S.L. (2004) Parasitoid coexistence: linking spatial field patterns with mechanism. *Ecology*, **85**, 667–678. <https://doi.org/10.1890/02-0566>.
- Briggs, C.J. (1993) Competition among parasitoid species on a stage-structured host and its effect on host suppression. *American Naturalist*, **141**, 372–397.
- Briggs, C.J. & Collier, T.R. (2001) Autoparasitism, interference, and parasitoid-pest population dynamics. *Theoretical Population Biology*, **60**, 33–57.
- De Bach, P. (1943) The importance of host-feeding by adult parasites in the reduction of host populations. *Journal of Economic Entomology*, **36**, 647–658.
- Flanders, S.E. (1942) *Metaphycus helvolus*, an encyrtid parasite of the black scale. *Journal of Economic Entomology*, **35**, 690–698.
- Frago, E. (2016) Interactions between parasitoids and higher order natural enemies: intraguild predation and hyperparasitoids. *Current Opinion in Insect Science*, **14**, 81–86.
- Goulet, H. & Hulent, H.F. (1993) *Hymenoptera of the World: An Identification Guide to Families. Research Branch, Agricultural. Canada Publication Canada Communication*. Group Publishing, Ottawa, Canada.
- Guerrieri, E. & Noyes, J.S. (2000) Revision of European species of genus *Metaphycus* Mercet (hymenoptera: Chalcidoidea: Encyrtidae), parasitoids of scale insects (Homoptera: Coccoidea). *Systematic Entomology*, **25**, 147–222. <https://doi.org/10.1046/j.1365-3113.2000.00099.x>.
- Hunter, M.S. & Woolley, J.B. (2001) Evolution and behavioural ecology of heteronomous aphelinid parasitoids. *Annual Review of Entomology*, **46**, 251–290.
- Hunter, M.S., Collier, T.R. & Kelly, S.E. (2002) Does an autoparasitoid disrupt host suppression provided by a primary parasitoid? *Ecology*, **83**, 1459–1469.
- Janssen, A., Montserrat, M., HilleRisLambers, R., Roos, A.M., Pallini, A. & Sabelis, M.W. (2006) Intraguild predation usually does not disrupt biological control (pp. 21–44). *Trophic and Guild in Biological Interactions Control*. Springer, Dordrecht, the Netherlands.
- Kapranas, A. & Luck, R.F. (2008) Egg maturation, host feeding, and longevity in two *Metaphycus* species parasitoids of soft scale insects. *Biological Control*, **47**, 147–153.
- Kapranas, A. & Tena, A. (2015) Encyrtid parasitoids of soft scale insects: biology, behaviour and their use in biological control. *Annual Review of Entomology*, **60**, 195–211.
- Kapranas, A., Morse, J.G., Pacheco, P., Forster, L.D. & Luck, R.F. (2007) Survey of brown soft scale *Coccus hesperidum* L. parasitoids in southern California citrus. *Biological Control*, **42**, 288–299.
- Luck, R.F. & Podoler, H. (1985) Competitive exclusion of *Aphytis lingnanensis* by *A. melinus*: potential role of host size. *Ecology*, **66**, 904–913.
- Manly, B.F.J., McDonald, L., Thomas, D.L., McDonald, T.L. & Erickson, W.P. (2002) *Resource Selection by Animals: Statistical Design and Analysis for Field Studies*. Cambridge University Press, Kluwer Academic Publishers, Dordrecht, the Netherlands.
- May, R.M. & Hassell, M.P. (1981) The dynamics of multiparasitoid-host interactions. *American Naturalist*, **117**, 234–261.
- Muegge, M.A. & Lambdin, P.L. (1989) Longevity and fecundity of *Coccophagus lycimnia* (walker) (hymenoptera: Aphelinidae), a primary parasitoid of *Coccus hesperidum* (Homoptera: Coccidae). *Journal of Agricultural Entomology*, **6**, 169–174.
- Murdoch, W.W., Chesson, J., & Chesson, P.L. (1985) Biological control in theory and practice. *The American Naturalist*, **125**, 344–366.
- Murdoch, W.W., Swarbrick, S.L., Luck, R.F., Walde, S. & Yu, D.S. (1996) Refuge dynamics and metapopulation dynamics: an experimental test. *The American Naturalist*, **147**, 424–444.
- Pekas, A., Tena, A., Harvey, J.A., Garcia-Marí, F. & Frango, E. (2016) Host size and spatiotemporal patterns mediate coexistence of specialist parasitoids. *Ecology*, **97**, 1345–1356.
- Pereira, J.A.C. (2004) *Bioecologia da cochonilha negra Saissetia oleae (Olivier), na oliveira, em Trás-os-Montes*. PhD dissertation, Universidade de Trás-os-Montes e alto Douro, Vila Real.
- Polis, G.A., Myers, C.A. & Holt, R.D. (1989) The ecology and evolution of the intraguild predation – potential competitors that eat each other. *Annual Review of Ecology and Systematics*, **20**, 297–330.
- Rosenheim, J.A. (1998) Higher-order predators and the regulation of insect herbivore populations. *Annual Review of Entomology*, **43**, 421–447.
- Rosenheim, J.A., Kaya, H.K., Ehler, L.E., Marois, J.J. & Jaffee, B.A. (1995) Interguild predation among biological-control agents: theory and evidence. *Biological Control*, **5**, 303–335.
- Schweizer, H., Morse, J.G., Luck, R.F. & Forster, L.D. (2002) Augmentative releases of a parasitoid (*Metaphycus* sp. nr. *flavus*) against citricola scale (*Coccus pseudomagnoliarum*) on oranges in the San Joaquin Valley of California. *Biological Control*, **24**, 153–166.
- Schweizer, H., Luck, R.F. & Morse, J.G. (2003) Augmentative releases of *Metaphycus* sp. nr. *flavus* against citricola scale on oranges in the San Joaquin Valley of California: are early releases better than late ones? *Journal of Agricultural Entomology*, **96**, 1375–1387.
- Snyder, W.E. & Ives, A.R. (2008) Behaviour influences whether intra-guild predation disrupts herbivore suppression by parasitoids. *Behavioural Ecology of Insect Parasitoids* (ed. by E. Wajnberg, C. Bernstein and J. van Alphen), pp. 71–91. Blackwell Press, Oxford, U.K.
- Snyder, R.E., Borer, E.T. & Chesson, P. (2005) Examining the relative importance of spatial and nonspatial coexistence mechanisms. *The American Naturalist*, **166**, 75–94.
- Tena, A. & Garcia-Marí, F. (2008) Suitability of citricola scale *Coccus pseudomagnoliarum* (Hemiptera: Coccidae) as host of *Metaphycus*

- helvolus* (hymenoptera: Encyrtidae): influence of host size and encapsulation. *Biological Control*, **46**, 341–347.
- Tena, A. & Garcia-Marí, F. (2009) Brood size, sex ratio and egg load of *Metaphycus lounsburyi* (hymenoptera: Encyrtidae) when parasitizing adult females of black scale *Saissetia oleae* (Hemiptera: Coccidae) in the field. *Biological Control*, **51**, 110–115.
- Tena, A., Soto, A., Vercher, R. & Garcia-Marí, F. (2007) Density and structure of *Saissetia oleae* (Hemiptera: Coccidae) populations on citrus and olives: relative importance of the two annual generations. *Environmental Entomology*, **36**, 700–706.
- Tena, A., Soto, A. & Garcia-Marí, F. (2008a) Parasitoid complex of black scale *Saissetia oleae* on citrus and olives: parasitoid species composition and seasonal trend. *BioControl*, **53**, 73–487.
- Tena, A., Kapranas, A., Garcia-Marí, F. & Luck, R.F. (2008b) Host discrimination, superparasitism and infanticide by a gregarious endoparasitoid. *Animal Behaviour*, **76**, 789–799.
- Tougeron, K. & Tena, A. (2019) Hyperparasitoids as new targets in biological control in a global change context. *Biological Control*, **130**, 164–171.
- Zang, L.S., Liu, T.X. & Wan, F.H. (2011) Reevaluation of the value of Autoparasitoids in biological control. *PLoS One*, **6**, e20324.

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